NEPHROGENIC DIABETES INSIPIDUS WITH A V2 RECEPTOR MUTATION AT A FUNCTIONALLY CRITICAL SITE

TOSHIYA NISHIKUBO, YORIKO KISATO, NAOKO ISHIKAWA, ISAO KUWAHARA and HIDEKAZU KAMITSUJI
Neonatal Intensive Care Unit, Nara Prefectural Hospital

KAZUAKI YOSHIZATO and EIICHI ARAKI
Department of Metabolic Medicine, Kumamoto University School of Medicine
Received February 21, 2005

Abstract : We present an infant with congenital nephrogenic diabetes insipidus with a V2 receptor mutation. The infant was admitted to our hospital because of high fever 7 days after birth. Laboratory examinations demonstrated elevated serum sodium (152 mEq/L) and chloride (113 mEq/L) concentrations. Plasma osmolarity was increased to 312 mOsm/kg, even though urinary osmolarity was 76 mOsm/kg. Serum AVP concentration was markedly increased. Pitressin-loading test did not show marked increment in the urinary osmolarity or secretion of urinary cyclic-AMP. Therefore, the patient was clinically diagnosed as having nephrogenic diabetes insipidus. DNA analysis of the V2 receptor gene identified a C to A transition at nucleotide position 1503. This mutation leads to a predictable change from Proline to Histidine. The patient was fed a sodium-restricted formula and administered trichlormethiazide. After initiating treatment, the serum concentration of Na was maintained below 150 mEq/L, and there was no physical dysfunction or mental retardation.

Early diagnosis followed by early interventions was important to improve the prognosis.

Key words : infant, nephrogenic diabetes insipidus, vasopressin type 2 receptor gene mutation

INTRODUCTION

Nephrogenic diabetes insipidus (NDI) involves the inability of the renal tubule to concentrate urine in response to arginine vasopressin (AVP). To date, mutations of the vasopressin type 2 receptor (V2R) gene or aquaporin 2 (AQP2) gene have been confirmed to cause NDI, and approximately 90 % of NDI cases are due to mutations of the V2R gene[1]. The V2R gene is localized in Xq28, and NDI with the V2R gene mutation shows an X-linked recessive inheritance (MIM 304,800)[2, 3]. AQP2 is a functional AVP-sensitive water channel, and AQP2 gene mutation shows either an autosomal recessive or dominant trail (OMIM 222,000 and 125,800, respectively)[4].

The clinical characteristics of NDI consist of severe polyuria and polydipsia in adults. In infants, non-specific episodes associated with water loss (fever, vomiting and diarrhea) are

often the main symptoms and delayed diagnosis is correlated with severe dehydration and hypernatremia causing seizure, mental retardation and cerebral calcification[5]. Early intervention with sodium restriction and diuretics is important to avoid these complications.

We report a congenital NDI patient diagnosed soon after birth by high fever and identified a missense mutation in the V2R gene.

CASE REPORT

The infant was born at 38 weeks of gestation after an uncomplicated pregnancy. Birth weight was 2,070g. He was admitted to our hospital because of high fever 7 days after birth. Family history included an uncle with nephrogenic diabetes insipidus. Body temperature was 38.1℃ on admission and skin turgor was slightly reduced. Heart rate was 164 times / minute and blood pressure was 62/40 mmHg. The large fontanel was not bulging.

Laboratory examinations demonstrated elevated serum sodium and chloride concentrations (Table 1). Serum osmolarity was increased, but urine osmolarity was low. Serum AVP concentration was markedly increased. Abdominal echography demonstrated normal renal structures.

Water restriction test did not demonstrate the elevation of urinary osmolarity over 150 mOsm/kg with high serum osmolarity at 310 mOsm/kg, and urinary osmolarity did not exceed the serum osmolarity. Pitressin-loading test did not show marked increment in the urinary osmolarity or secretion of urinary cyclic-AMP (Fig.1). Therefore, the patient was clinically diagnosed as having NDI and the presence of an abnormality in vasopressin type 2 receptor was suggested due to the X-linked transmission of the phenotype and the loss of the secretary elevation of urinary cyclic-AMP.

Table 1. Laboratory data of the proband on admission

Peripheral blood			**Urinary analysis**		
RBC	540	$\times 10^4/\mu L$	Specific Gravity	1.003	
Hb.	17.9	g/dL	Protein	(−)	
Ht.	56.7	%	Glucose	(−)	
Plt.	34	$\times 10^4/\mu L$	Ketone body	(−)	
WBC	14,100	$/\mu L$	Sediment	n.p.	
Blood chemistry			**Urine Biochemistry**		
CRP	0.2	mg/dL	Urine osmolarity	76	mOsm/kg
BUN	11	mg/dL	Na	22	mEq/L
Cr.	0.5	mg/dL	Cl	13	mEq/L
Na	152	mEq/L	Cr.	7.5	mg/dL
K	5.7	mEq/L	NAG	16.1	IU/L
Cl	113	mEq/L	β_2-MG	5744	$\mu g/L$
Plasma osmolarity	312	mOsm/kg	FENa	0.93	%
Hormonal analysis					
AVP	78	pg/mL			

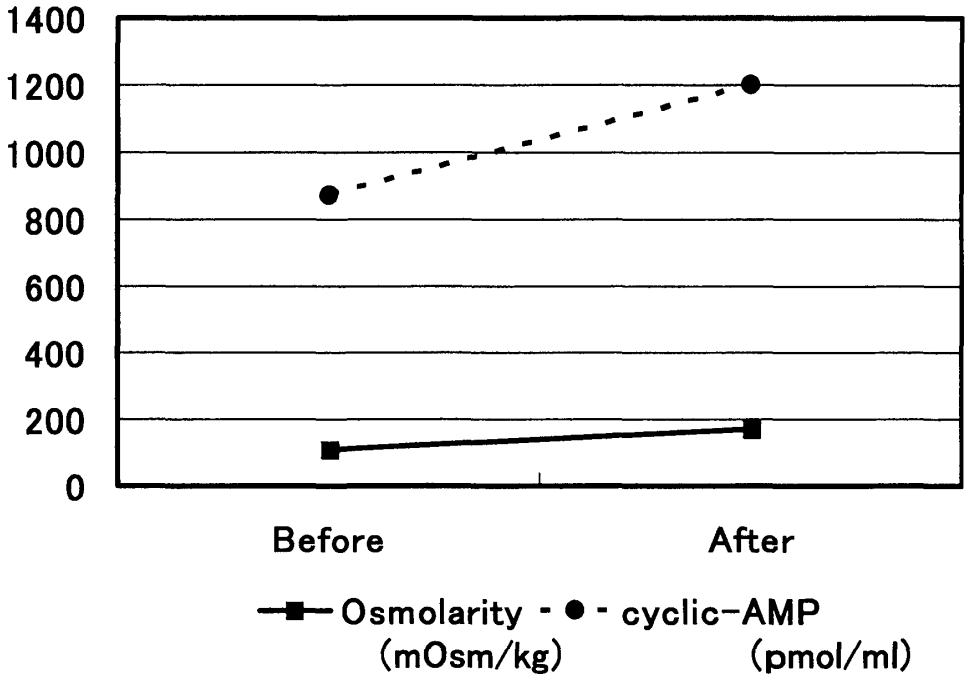


Fig. 1. Pitressin - loading test. Urinary osmolarity and the secretion of urinary cyclic-AMP did not increase markedly after the administration of pitressin.

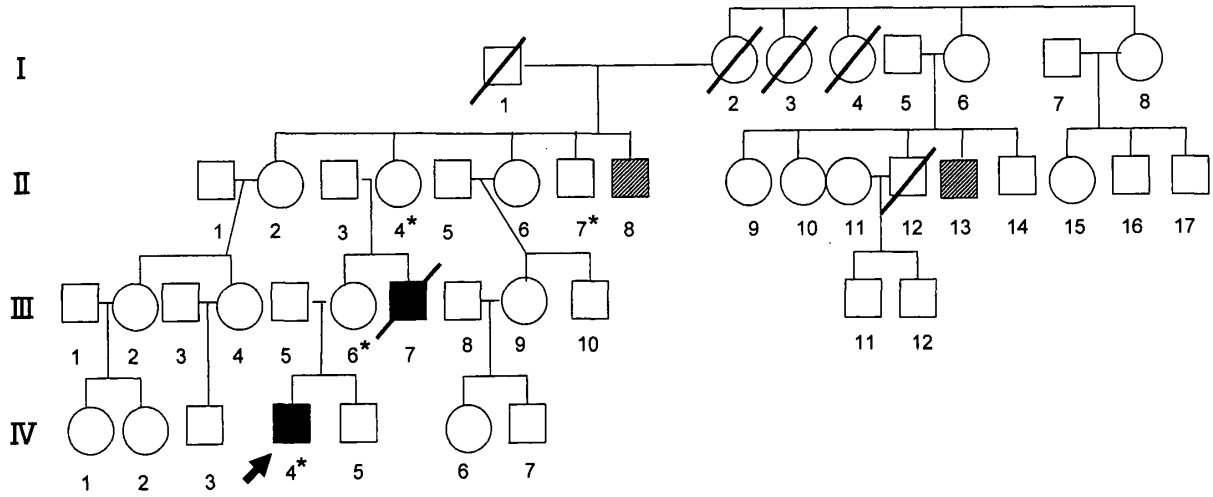


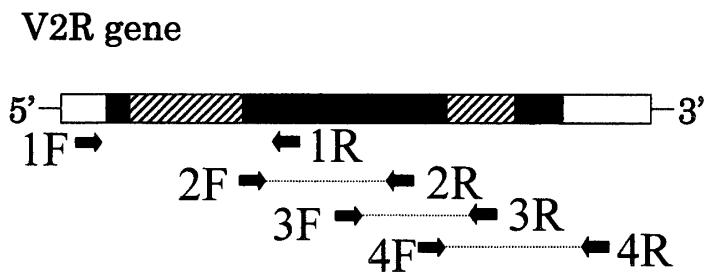
Fig. 2. Pedigree of the NDI family. Black symbols denote affected individuals. Hatched symbols indicate those in whom NDI was suspected. Symbols with diagonal slashes denote deceased individuals. Those whose samples were analyzed by direct sequencing of PCR products are denoted by asterisks.

The pedigree of the family is shown in Fig. 2. Results of laboratory examinations of the relatives are shown in Table 2. Serum and urinary osmolarities were normal in all relatives, but serum AVP was increased in the mother (Ⅲ-6) and grandmother (Ⅱ-4). DNA analysis of V2R gene was performed as followed after informed consent was obtained from parents and relatives.

Table 2. Characterization of laboratory data in the indviduals

No of pedegree	Ⅳ-4	Ⅱ-4	Ⅱ-7	Ⅲ-6	Ⅳ-5
Blood chemistry					
BUN (mg/dL)	11	8	7	8	2
Cr. (mg/dL)	0.5	0.6	0.6	0.4	0.5
Na (mEq/L)	152	144	142	140	136
K (mEq/L)	5.7	3.7	4.5	3.7	4.3
Cl (mEq/L)	113	107	104	107	110
Osmolarity (mOsm/kg)	312	290	290	280	274
Hormonal analysis					
AVP (pg/mL)	78	22.5	2.8	8.1	12.6
Urinary analysis					
Osmolarity (mOsm/kg)	76	322	441	331	91

a)



b)

1F;-52:5'-CTGAGTCCGCACATCACC-3':-35 1R; 150:5'-AGCCACAAAGACTATGGA-3':133
2F; 33:5'-TGGGCATCCCTCTCTGCC-3': 5 2R; 537:5'-GGCGAAGATGAAGAGCTG-3':520
3F;426:5'-CCGTCCCATGCTGGCGTA-3':443 3R; 906:5'-CAGAGGTGCCTCCGGGTC-3':889
4F;780:5'-CCACGTGTCAGCAGCTGT-3':797 4R;1193:5'-CAGTGGCACCCAGGACCA-3':1176

Fig. 3. a) Schematic representation of the V2R gene with the locations of the primers indicated by the arrows. Black boxes denote the coding regions and open boxes indicate 5' and 3' non - coding regions of the V2R gene. Hatched boxes represent introns.
b) 1F-4F and 1R-4R represent the positions and sequences of forward (F) and reverse (R) primer sets used for PCR to generate the fragment to analyze the V2R gene. Numbers before and after primer sequences indicate nucleotide positions of the V2R gene.

GENOMIC V2R DNA AMPLIFICATION AND GENE SEQUENCING

Genomic DNA was extracted from peripheral white blood cells from the proband (Ⅱ-4) and their relatives (Ⅱ-4, Ⅱ-7, Ⅲ-6) with a DNA isolation kit (QIAamp Blood Kit; QIAGEN, Dusseldorf, Germany). Three coding exons of the V2R gene were amplified by PCR with oligonucleotide primers as previously described[6] (Fig. 3). Amplification was performed with *Taq* polymerase, and amplified products were purified on 1.5% agarose gels before sequencing. Direct sequencing was performed by the dideoxy method using a sequencing system (CEQ2000 Dye Terminator Cycle Sequencing with Quick Start Kit; BECKMAN COULTER INC., Fullerton, CA) and an automatic DNA sequencer (CEQ 2000XL; COULTER INC., Fullerton, CA).

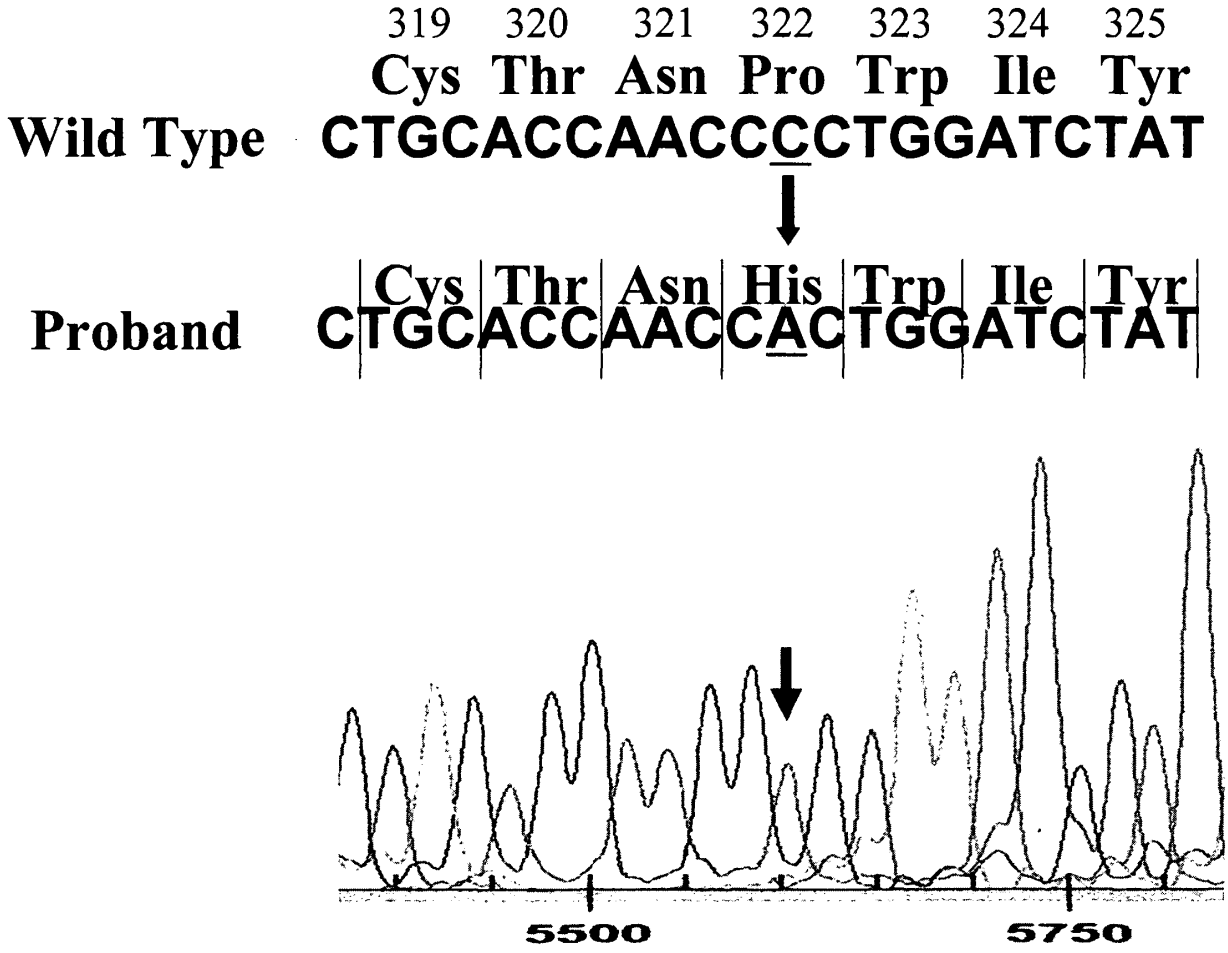


Fig. 4. The result of direct sequencing of the V2R gene of the propositus. Missense mutation of C to A transition at nucleotide position 1503 in exon 3 leads to a predicted change of Proline (codon 322, CCC) to a Histidine (CAC).

Direct sequencing of the V2R gene of the propositus identified a C to A transition at nucleotide position 1503 in exon 3 (Fig. 4). This mutation leads to a predictable change from Proline (codon 322, CCC) to Histidine (CAC). This missense mutation is located within the seventh transmembrane domain of the V2R gene, named P322H. The patient's mother (Ⅲ-6) and grandmother (Ⅱ-4) carried the same affected X chromosome but had another X chromosome without P322H. An A to G single nucleotide polymorphism at nucleotide 1465 was also identified in this patient (hemizygous), mother (heterozygous) and grandmother (homozygous), but was not found in an unaffected male relative (Ⅱ-7) (data not shown).
The patient was fed a sodium-restricted formula and administered trichlormethiazide to improve hypernatremia and polyuria. His fluid intake was 250 to 300 ml/kg/day and the serum concentration of sodium was kept below 145 mEq/L after the commencement of these therapies. The patient was discharged at the age of 50 days.

After discharge, the patient presented with recurrent vomiting. Growth retardation continued at -4 SD both in weight and in height at one year and 4 months old. However, the serum concentration of Na was maintained below 150 mEq/L, and there was no physical dysfunction or mental retardation. Abdominal echography did not detect any abnormal

findings in the bilateral kidneys or bladder.

DISCUSSION

Infants complicated with NDI demonstrate non-specific signs, such as high fever, which also appeared in our patient. However, events associated with failure to thrive due to severe dehydration could be the initial symptom of NDI. Van Lieburg et al. reported that symptoms associated with failure to thrive were the first basis for referral in 50% of infants with NDI. Therefore, early diagnosis with adequate treatment is necessary to improve the prognosis. However, the mean age at diagnosis was reported to distribute to 25 ± 44 months[5], suggesting the difficulty in early diagnosis of NDI. Our patient was diagnosed as having NDI soon after birth due to information on the presence of NDI relatives. Thus, irreversible damage including mental impairment was prevented.

Because of the X-linked transmission of the phenotype and blunted response of urinary cyclic-AMP upon pitressin-loading test, abnormality of the V2R gene was suggested in the proband. In our case, a missence mutation of P322H located in the seventh transmembrane domain of V2R gene was identified. This mutation was noted previously in another Japanese NDI patient unrelated with to the present case[7].

Interestingly, this Pro322 amino acid has been reported to be important for receptor function. Expression of the P322H mutant of V2R gene in COS-7 cells showed weak but specific binding to AVP but failed to stimulate adenylyl cyclase activity. However, another point mutation of P322S found in NDI patients with a mild clinical phenotype was able to partially stimulate adenylyl cyclase activity. Three dimensional modeling of the mutant receptors suggested that complete loss of function of the P322H could be due to hydrogen bond formation between His322 side chain and carboxyl group of Asp85, which does not occur in the P322S variant[8].

To date, more than 100 different types of mutations, insertions, or deletions in the gene have been reported[9]. However, there was no relationship reported between locations of the mutations of V2R gene and clinical characteristics in NDI patients except for P322 and G185 mutations[5]. G185C mutation is located in the second extracellular loop of the V2R receptor. Mutations in this loop other than G185C cause the complete clinical phenotype[10, 11]. However, the G185C mutation causes a partial NDI phenotype, and the patients with this mutation tend to be diagnosed in adulthood or remain undiagnosed. *In vitro* expression study of G185C has not been reported.

In the relatives, the grandmother and mother carried identical mutations detected by DNA analysis, and laboratory examination indicated the elevation of their serum AVP concentrations. Skewed X-inactivation explained these laboratory investigations in the elevation of AVP in these female cases. Affected female NDI patients were reported by van Lieburg et al[12].

Long-term follow-up including medication, sodium restriction, growth monitoring and early detection of complications such as hydronephrosis are also important for an NDI infant[13]. In our case, there were no complications other than growth restriction. Despite early treatment with thiazide, growth retardation until one year after birth might be inevitable in an infant with NDI. However, it has been reported that catch-up growth occurs after the first

year of age and generally normalizes by school age[5]. In addition, our patient was small for date at birth, and this factor may have amplified the growth restriction of the patient. Mental retardation and cognitive dysfunction are considered important complications of NDI. Further investigation is necessary for follow-up in NDI patients[14].

REFERENCE

1) **Morello, J. P.** and **Bichet, D. G.** : Nephrogenic diabetes insipidus. Annu. Rev. Physiol. **63** : 607-630, 2001.
2) **van den Ouweland, A. M., Knoop, M. T., Knoers, V. V., Markslag, P. W., Rocchi, M., Warren,S.T., Ropers, H. H., Fahrenholz, F., Monnens, L. A.** and **van Oost, B. A.** : Colocalization of the gene for nephrogenic diabetes insipidus (DIR) and the vasopressin type 2 receptor gene (V2R) in the Xq28 region. Genomics **13**:1350-1352, 1992.
3) **Seibold, A., Brabet, P., Rosenthal, W. and Birnbaumer, M.** : Structure and chromosomal localization of the human antidiuretic hormone receptor gene. Am. J. Hum. Genet. **51**:1078-1083, 1992.
4) **Sasaki, S., Fushimi, K., Saito, H., Saito, F., Uchida, S., Ishibashi, K., Kuwahara, M., Ikeuchi, T., Inui, K., Nakajima, K., Watanabe, T.** and **Marumo, F.** : Cloning, characterization, and chromosomal mapping of human aquaporin of collecting duct. J. Clin. Invest. **93** : 1250-1256, 1994.
5) **van Lieburg, A. F., Knoers, N. V.** and **Monnens, L. A.** : Clinical presentation and follow-up of 30 patients with congenital nephrogenic diabetes insipidus. J. Am. Soc. Nephrol. **10** : 1958-1964, 1999.
6) **Jinnouchi, H., Araki, E., Miyamura, N., Kishikawa, H., Yoshimura, R., Isami, S., Yamaguchi, K., Iwamatsu, H.** and **Shichiri, M.** : Analysis of vasopressin receptor type II (V2R) gene in three Japanese pedigrees with congenital nephrogenic diabetes insipidus: identification of a family with complete deletion of the V2R gene. Eur. J. Endocrinol. **134** : 689-698, 1996.
7) **Tajima, T., Nakae, J., Takekoshi, Y., Takahashi, Y., Yuri, K., Nagashima, T., Fujieda, K.** : Three novel AVPR2 mutations in three Japanese families with X-linked nephrogenic diabetes insipidus. Pediatr. Res. **39** : 522-526, 1996.
8) **Ala, Y., Morin, D., Mouillac, B., Sabatier, N., Vargas. R., Cotte, N., Dechaux, M., Antignac, C., Arthus, M. F., Lonergan, M., Turner, M. S., Balestre, M. N., Alonso, G., Hibert, M., Barberis, C., Hendy, G. N., Bichet, D. G.** and **Jard, S.** : Functional studies of twelve mutant V2 vasopressin receptors related to nephrogenic diabetes insipidus: molecular basis of a mild clinical phenotype. J. Am. Soc. Nephrol. **9** : 1861-1872, 1998.
9) **Birnbaumer M.** : Vasopressin receptor mutations and nephrogenic diabetes insipidus. Arch. Med. Res. **30** : 465-474, 1999.
10) **Yokoyama, K., Yamauchi, A., Izumi, M., Itoh, T., Ando, A., Imai, E., Kamada, T.** and **Ueda, N.** : A low-affinity vasopressin V2-receptor gene in a kindred with X-linked nephrogenic diabetes insipidus. J. Am. Soc. Nephrol. **7** : 410-414, 1996.
11) **Pan, Y., Wilson, P. and Gitschier, J.** : The effect of eight V2 vasopressin receptor mutations on stimulation of adenylyl cyclase and binding to vasopressin. J. Biol. Chem. **269** : 31933-31937, 1994.
12) **van Lieburg, A. F., Verdijk, M. A., Schoute, F., Ligtenberg, M. J., van Oost, B. A., Waldhauser, F., Dobner, M., Monnens, L. A.** and **Knoers, N. V.** : Clinical phenotype of nephrogenic diabetes insipidus in females heterozygous for a vasopressin type 2 receptor mutation. Hum. Genet. **96** : 70-78, 1995.
13) **Ten Bensel, R. W., Peters, E. R.** : Progressive hydronephrosis, hydroureter, and dilatation of the bladder in siblings with congenital nephrogenic diabetes insipidus. J. Pediatr. **77** : 439-443, 1970.
14) **Hoekstra, J. A., van Lieburg, A. F., Monnens, L. A., Hulstijn_Dirkmaat, G. M. and Knoers, V. V.** : Cognitive and psychosocial functioning of patients with congenital nephrogenic diabetes insipidus. Am. J. Med. Genet. **61** : 81-88, 1996.